

Technical Progress Report

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During the period covered by this report, the development of mass spectrometric methods for the detection and characterization of traces of organic compounds in terrestrial and extraterrestrial materials has been continued.

Emphasis was placed on the design of instrumentation; development of procedures for obtaining the mass spectra; development of computer techniques to treat and interpret the data and, finally, testing the procedures on terrestrial and extraterrestrial samples (meteorites).

Based on previous work from our laboratory (in part carried out with the support of NASA grant NsG 211-62), mass spectrometry seems to have emerged as the most promising technique for the investigation of terrestrial or extraterrestrial material for organic compounds, mainly because of two of its attributes: First, it is most generally applicable since any molecule has a specific mass and the interpretation of the data does not depend on comparison with a known system. Second, it is extremely sensitive, presently requiring only fractions of a microgram. This range certainly could be pushed downwards a few orders of magnitude if need be.

These two aspects are extremely important for the problem at hand, since one has to be prepared to find substances that

differ from those known on earth and which also may be present in extremely small quantities. The only limitation of mass spectrometry is the necessity of a certain minimum vapor pressure ($>10^{-6}$ mm) and thermal stability. This eliminates work on polymeric or highly polar compounds as such, but ways can be found (pyrolysis, chemical conversion) to make these amenable too. Fortunately, in the exploration of a completely new system, such as another planet, the compounds exhibiting a sufficient vapor pressure would anyway be of extreme interest and be representative of the vast majority.

It should again be stressed that the mass spectra are directly related to chemical structure and this independence from the availability or even existence of comparison samples is one of the most important advantages of mass spectrometry in this field. It is even not restricted to the type of "organic" molecules we are used to, because the preponderance of the elements C, H, N, O, S, etc. is not a prerequisite. For example, the spectrum of a silicone compound in which the "chain" is not C-C-C.... but Si-O-Si-O-Si.... has groups of peaks 74 mass units rather than 14 mass units apart ($(\text{CH}_3)_2\text{SiO}$ rather than CH_2). Furthermore, it exhibits the typical triplet pattern of peaks, due to the appreciable abundance of the heavy silicone isotopes (^{29}Si and ^{30}Si).

In the simplest case (low organic content, consisting of simple compounds) the finely ground specimen is placed directly into the ion source of the mass spectrometer and slowly heated. Spectra are recorded continuously during the vaporization of the organic materials present in the sample. This technique gives good results (see the later example, Holbrook meteorite) but for

substances richer in organics too complex spectra are obtained.

In such cases some fractionation has to be accomplished in order to obtain a set of spectra of simpler mixtures, or, ideally, pure compounds.

Beyond crude thermal fractionation (controlled vaporization), gas chromatography offers properties which single it out from all other separation techniques. It is unique in its compatibility with mass spectrometry. It is a highly sensitive technique, and its limitations are of the same types as those of mass spectrometry: volatility and thermal stability of the sample. The volatility requirements are somewhat more severe for gas chromatography, and this assures that any compound which passes through a gas chromatographic column is amenable to mass spectrometry.

A technique had been previously developed¹ and was further improved during this grant period, which permits the direct connection of a gas chromatograph to the mass spectrometer. This technique, the details of which will be discussed later in connection with an actual example, permits the recording of the mass spectra of individual components of a mixture. In very complex systems even gas chromatographic separation may not be complete, but fractions obtainable consist then at most of a few components and the resulting spectrum is still interpretable.

Our work on high-resolution mass spectrometry has been continued to obtain much more detailed mass spectrometric data, namely the elemental compositions of the ions rather than merely their nominal mass. The advantages are obvious: the spectra of mixtures can be interpreted with much greater certainty, and the elements present are revealed. The penalty for this increase in

information content is the increased complexity of the data (six significant numbers for each mass instead of three). The efficient use of this more complete information requires a computer^{2,3}

The technique was first tested quite exhaustively with terrestrial sediments and then applied to two meteorite samples⁴. One of these has a very low content of organic material and was selected as an example showing the utility of the technique as a speedy survey test for quantity and type of organics present. The second one (Murray) contained more and was used to represent a case where more detailed information on the larger amounts of organic compounds are desired and obtainable. Both samples represented specimens of a longitudinal core drilled through a stone. Only one core segment of each is discussed in this progress report. Details were presented at a number of specialized meetings⁵.

As a representative of a material containing very little organic material a sample of Holbrook meteorite (a non-carbonaceous chondrite) was examined. About 0.100g of powdered material representing an internal region of a core drilled through the stone were carefully placed near the ionizing region of the ion source of the high resolution mass spectrometer. After evacuation of the system, the sample was slowly heated while constantly recording the ions produced by the electron beam from any material vaporizing out of the sample at that temperature and pressure (10^{-6} mm).

The top of Fig. 1 represents the mass spectrum (in terms of the elemental composition of the ions formed³) of the products evolved at temperatures between 25 and 153°C. Above mass 50 (the lower limit of this run) only two hydrocarbon ions

($C_4H_7^+$ and $C_6H_6^+$) are found, and these are of very low abundance (the asterisks represent $2.7 \times \log$ of relative abundance). One species containing oxygen ($C_3H_6O^+$) and another containing sulfur ($C_5H_4S^+$) are also recorded (X stands for S in Figs. 1 and 3). These represent extremely small traces of material, indicating that practically nothing emanates in this range.

At higher temperatures (153 - 300°C, bottom of Figure 1) evolution of carbon containing material becomes quite pronounced. Examination of the compositions of the hydrocarbon ions indicates the presence mainly of hydrocarbons of various degrees of saturation and up to six or seven carbon atoms as well as aromatic systems. The latter are indicated by the high abundance of $C_6H_6^+$ (benzene), $C_7H_8^+$ (toluene), $C_8H_{10}^+$ (xylene or ethylbenzene) and $C_9H_{12}^+$ (trimethylbenzene or isomer). In addition to these, there are oxygenated compounds, most of which have a high hydrogen content and which represent ketones (e.g. acetone, C_3H_6O , methyl ethyl ketone, C_4H_8O etc.) with possibly small amounts of aromatic oxygenated materials ($C_7H_5O^+$, benzoyl from benzoic acid?) present. The sulfur containing ions (CHX represents CHS) are of low hydrogen content and thus aromatic (thiophenes). It should be noted that nitrogen containing species are absent, and so are any other ions containing nitrogen, sulfur and up to three oxygens.

It is obvious that this simple experiment reveals the presence of a variety of organic compounds in a material previously thought to contain none at all. The very low abundance and relatively simple compositions makes it unnecessary or even undesirable to achieve separation of the components prior to their reaching the ion source of the spectrometer.

Under the same conditions the much larger amount of "organic" material present in a class II carbonaceous chondrite gave a highly complex spectrum, indicating the need for simplification to a set of less complex systems.

The scarcity of the material as well as the danger of contamination with terrestrial substances requires direct coupling of the vaporization, separation, and mass spectral steps without intermediate handling of the sample.

The equipment schematically illustrated in Fig. 2 is used for this purpose⁴. The powdered sample is placed in the furnace in a helium stream. The emanating vapors are carried into a cooled U-tube and condensed. After 10-30 minutes the U-tube is heated suddenly to 300°, flushing the condensate onto the gas chromatographic column for separation. The carrier gas (and eluted fractions) emerging from the column enter the "helium separator", a vacuum jacketed fritted glass tube¹. Most of the helium passes through the walls, while the relatively heavier compound molecules are mostly carried through the tube and to the ion source. After ionization by the electron beam, the ions formed pass through the electrostatic field for velocity focusing and a small fraction of them strikes a collector at that point. The signal generated by this collector is used to plot the "chromatogram" much as any gas chromatographic detector does. The majority of the ions, however, enter the magnetic field. They are separated according to their mass while traversing this field and are recorded on a photographic plate placed in the focal plane. After each fraction the plate is advanced vertically by the height of the ion beam thus enabling us to record the complete spectra of sixty consecutive regions of

the gas chromatogram.

At the end of the experiment the plate is removed from the instrument, developed, and densities and positions of the recorded lines are measured by an automatic comparator generating the data in computer compatible form. To permit calculation of accurate masses, a mass calibration compound (fluorocarbon) is continuously fed into the ion source, giving rise to recognizable lines of exactly known mass. A sophisticated computer program accomplishes all the data processing and calculates the elemental compositions of all the ions formed⁶.

Figure 3 shows the gas chromatogram recorded when passing the material which vaporized (temperature range 136 - 250°C) out of 0.324g of powdered Murray meteorite (a type II carbonaceous chondrite). It clearly indicates a very complex mixture incompletely resolved even by the gas chromatograph. Twenty eight consecutive spectra were taken (as indicated by the vertical lines) and interpreted.

As an example, the spectrum of fraction 5 is shown in Fig. 4. The most abundant ions are $C_7H_7^+$, $C_8H_{10}^+$, $C_5H_5S^+$, $C_6H_7S^+$ and $C_6H_8S^+$. There is no doubt that these are due to the presence of xylene (or ethyl benzene, these particular isomers are very difficult to distinguish mass spectrometrically) and methyl thiophene. There are, however, some hydrocarbon ions of a hydrogen content higher than aromatics (e.g. $C_5H_9^+$, $C_7H_{13}^+$ and $C_8H_{13}^+$) corresponding to fragments of traces of mono- and/or diolefines with more than eight carbon atoms.

Similar analysis of the spectra obtained from the other fractions led to the tentative identifications of the compounds listed in

Table I. These results are quite remarkable, considering that they represent a single experiment on a third of a gram of meteorite (the volatiles represented by the chromatogram are probably in the order of a hundred micrograms).

The data are much too preliminary to embark on a speculation as the origin of these substances. While they are most likely indigenous it would be premature to say whether they are abiotic products or the result of living organisms. The lack of nitrogen containing ions is, however, surprising, as well as the apparently relatively high abundance of unbranched hydrocarbons (at least in the C_{12} - C_{20} region) as judged from the tall peaks in the chromatogram.

The above discussion points out that these techniques substantially advance our capability in detecting and characterizing minute amounts of organic substances in extraterrestrial material available on earth. It also permits us to extrapolate to what can be best achieved by a miniaturized instrument operated automatically and remotely. The construction of small mass spectrometers of a resolving power for distinguishing integral masses is within the state of the art and small automated gas chromatographs have been developed already some time ago in connection with the Surveyor program⁷. Their combination following the principles outlined above should be possible. The remaining problems to be solved concern the sample acquisition and the pumping system, not only for the operation of the mass spectrometer but also for the removal of the carrier gas.

The large amount of data generated, even by a spectrometer of moderate resolving power, might tax the telemetry capability

over large distances. Programmed on-site selection of the most useful portion of the data, or ultimately, automatic interpretation of the data and transmission of the results only would be a logical approach.

With this in mind, we are continuing our efforts⁸⁻¹¹ of direct computer interpretation of mass spectrometric data, in order to apply them to the interpretation of the spectra obtained from meteorite samples.

A more highly automated comparator has been developed¹² to cope effectively with the many hundreds of high resolution mass spectra recorded in the course of this work.

A paper describing this work which presently was only discussed at meetings^{5,12} is in preparation.

References

1. High-Resolution Mass Spectra of Compounds Emerging from a Gas Chromatograph, J.T. Watson and K. Biemann, Anal. Chem. 36, 1135 (1964); Direct Recording of High Resolution Mass Spectra of Gas Chromatographic Effluents, J.T. Watson and K. Biemann, Anal. Chem. 37, 844 (1965).
2. Techniques in the High-Resolution Mass Spectroetry of Complex, Polyfunctional Organic Molecules, P. Bommer, W. J. McMurray and K. Biemann, 12th Annual Conference on Mass Spectrometry, Montreal, June 1964; Computer Techniques for the Fast and Facile Conversion of Line Positions to Elemental Compositions of Ions Recorded on Photographic Plates, D. Desiderio and K. Biemann, *ibid.*; High-Resolution Mass Spectra of Organic Molecules and Their Use for Structure Determination, K. Biemann, W.J. McMurray and P. Bommer, *ibid.*
3. Element-Mapping, A New Approach to the Interpretation of High Resolution Mass Spectra, K. Biemann, P. Bommer and D.M. Desiderio, Tetrahedron Letters, 1725 (1964).
4. J.M. Hayes, Ph.D. Thesis, M.I.T., in preparation.
5. Papers presented at the meetings of the "Committee for the Analysis of Carbonaceous Chondrites", April, 1965 and April, 1966, Washington, D.C.; and the 12th Meeting of the American Astronomical Society, May 1966.

6. Dominic M. Desiderio, Ph.D. Thesis, M.I.T., December 1965.
7. W.F. Wilhite, "The Development of the Surveyor Gas Chromatograph," Technical Report No. 32-425, Jet Propulsion Laboratory, Pasadena, California, 1963.
8. Computer-Aided Interpretation of High Resolution Mass Spectra, K. Biemann and W.J. McMurray, Tetrahedron Letters, 647 (1965).
9. 'Ion-Types', A Useful Concept in the Interpretation of High-Resolution Mass Spectra, K. Biemann, W.J. McMurray and P.V. Fennessey, Tetrahedron Letters, 3997 (1966).
10. Computer-Aided Correlation of Metastable Peaks in High Resolution Mass Spectrometry, N. Mancuso, S. Tsunakawa and K. Biemann, Anal. Chem., in press.
11. Computer-Aided Interpretation of High Resolution Mass Spectra II. Amino Acid Sequence of Peptides, K. Biemann, C. Cone and B.R. Webster, J. Am. Chem. Soc. 88, 2597 (1966).
12. A Computer Compatible Comparator-Densitometer for Use in High Resolution Mass Spectrometry, K. Biemann, P.V. Fennessey and J.M. Hayes, presented at Meeting of the Society of Photo-Optical Instrumentation Engineers, Boston, June 1966.

ELEMENT MAP537-13-1 HOLBROOK CORE 4, 25-153 J.H.

	CH	CHN	CHO	CHNO	CHX
55	4/ 7 0**				
58			3/ 6 0**		
64					
78	6/ 6 0**				
96					5/ 4 2**

	CH	CHN	CHO	CHNO	CHX
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ELEMENT MAP537-14-1 HOLBROOK CORE 4, 153-300 J.H.

	CH	CHN	CHO	CHNO	CHX
52	4/ 4 0**				
53	4/ 5 0***				
54	4/ 6 0**				
55	4/ 7 0****		3/ 3 0****		
56	4/ 8 0****		3/ 4 0**		
57	4/ 9 1**		3/ 5 0****		
58			3/ 6 0*****		2/ 2 0*
60					
64	5/ 4 0*				
65	5/ 5 0***				
66	5/ 6 0***				
67	5/ 7 0***				
68	5/ 8 0**		4/ 4 0*		
69	5/ 9 0****		4/ 5 0**		
70	5/10 0***		4/ 6 0***		
71	5/11 0**		4/ 7 0**		
72			4/ 8 0****		
76	6/ 4 0**				
77	6/ 5-1****				
78	6/ 6 0*****				
79	6/ 7-1***				
80	6/ 8 1*				
81	6/ 9-1***		5/ 5-1**		
82	6/10 0**		5/ 6 1*		
83	6/11 0***				
84	6/12 1**				4/ 4 1**
85	6/13 2*				
86			5/10 1**		
91	7/ 7 0*****				
92	7/ 8 0*****				
93	7/ 9-1***				
95	7/11 0**				
96					5/ 4 2**
97					5/ 5 0**
102					
105	8/ 9 0****		7/ 5 0**		
106	8/10 0****				
117	9/ 9-1**				
119	9/11-1****				
120	9/12 0**				
124					
207					

	CH	CHN	CHO	CHNO	CHX
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Fig. 1.. High resolution mass spectrum (as elemental compositions) of organic materials in Holbrook chondrite (see text).

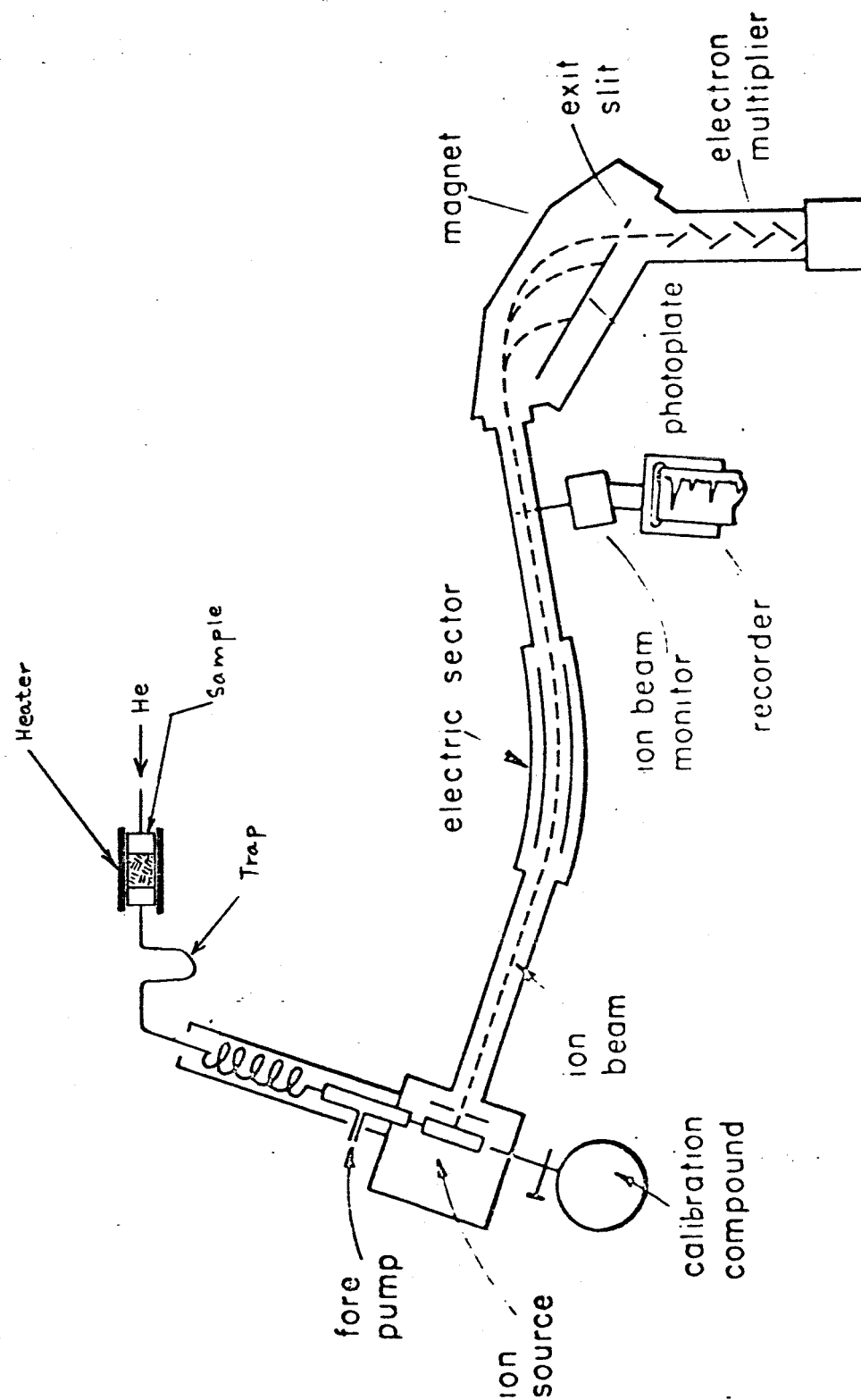


Fig. 2. Schematic of a high resolution mass spectrometer equipped for "pyrolysis" gas chromatography.

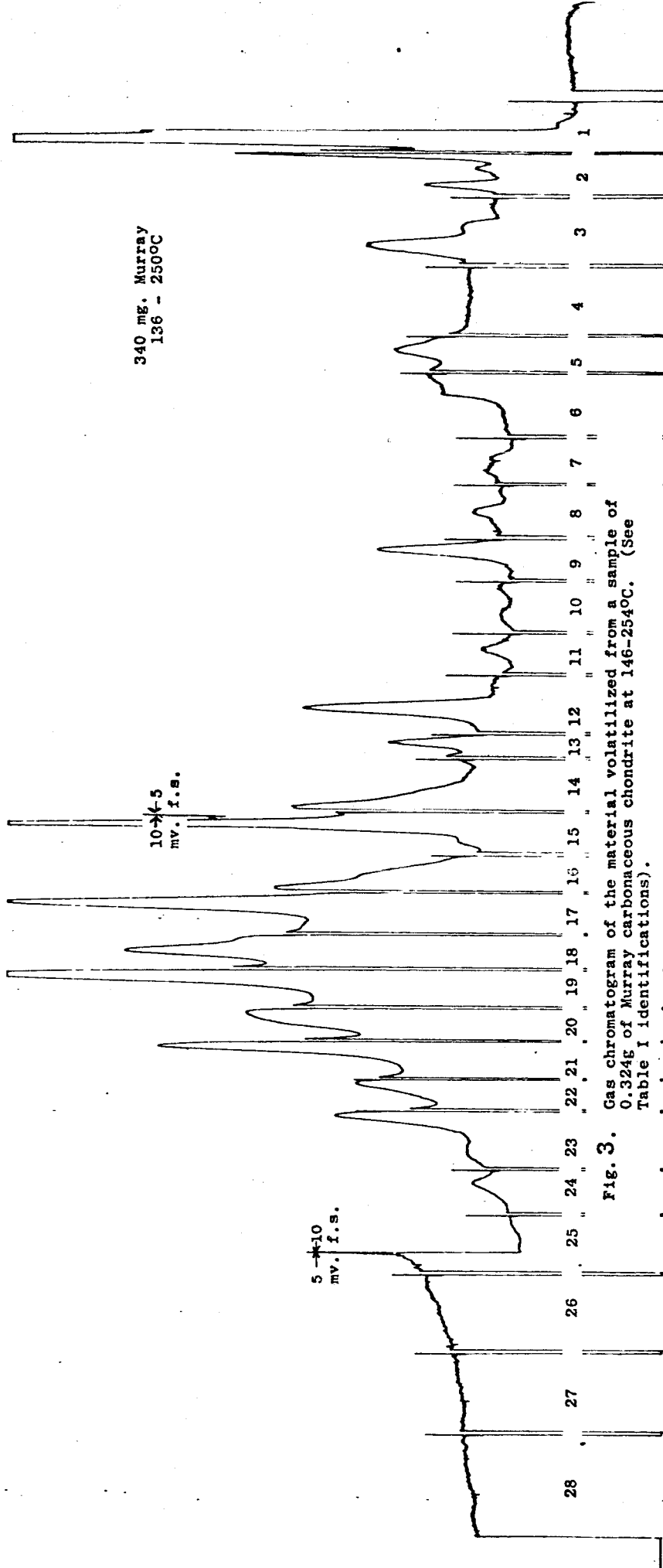


Fig. 3. Gas chromatogram of the material volatilized from a sample of 0.324g of Murray carbonaceous chondrite at 146-254°C. (See Table I identifications).

ELEMENT MAP532-30-1 MURRAY GLC-III-254-05 J.H.			
CHN	CHO	CHNC	CHX
CH	CHN	CHNO	CHNX
65 5/ 5 C***			
67 5/ 7 C*			
68 5/ 8 C*			
69 5/ 9-1***			
70 5/10 C*			3/ 3 0*
71 5/11 C**			
77 6/ 5 C***			
78 6/ 6 C***			
79 6/ 7 C**			
81 6/ 9 C*			
83 6/11-1*			4/ 4-2*
84			
85 7/ 1-2*			
87 7/ 3 C*			
89 7/ 5-2*			
91 7/ 7-1***			
95 7/11 C*			5/ 5 0*****
97 7/13 C**			
98 8/ 2 C*			
102 8/ 6 1**			
103 8/ 7 C***			
104 8/ 8 C***			
105 8/ 9 C***			
106 8/10 C***			
109 8/13 C*			6/ 6 0*
110			6/ 7 0*****
111			6/ 8 0*****
112			
113 9/ 5-1*			
119 9/11-1*			
126 9/18-1*			
CH	CHN	CHNO	CHX
CH	CHN	CHNO	CHNX

Fig. 4. High resolution mass spectrum (as elemental compositions) of Fraction 5 in Fig. 3.